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Response to dietary phosphorus deficiency is affected by genetic background in growing pigs¹

L. S. Alexander,*† A. Qu,* S. A. Cutler,* A. Mahajan,*† S. M. Lonergan,* M. F. Rothschild,*‡
T. E. Weber,§ B. J. Kerr,§ and C. H. Stahl†²

*Department of Animal Science, Iowa State University, Ames 50010; †Department of Animal Science, North Carolina State University, Raleigh, NC 27695; ‡Center for Integrative Animal Genomics, Iowa State University, Ames 50011; and §USDA-ARS, Ames, IA 50011

ABSTRACT: Concern over the environmental effect of P excretion from pig production has led to reduced dietary P supplementation. To examine how genetics influence P utilization, 94 gilts sired by 2 genetic lines (PIC337 and PIC280) were housed individually and fed either a P-adequate diet (PA) or a 20% P-deficient diet (PD) for 14 wk. Initially and monthly, blood samples were collected and BW recorded after an overnight fast. Growth performance and plasma indicators of P status were determined monthly. At the end of the trial, carcass traits, meat quality, bone strength, and ash percentage were determined. Pigs fed the PD diet had decreased ($P < 0.05$) plasma P concentrations and poorer G:F ($P < 0.05$) over the length of the trial. After 4 wk on trial, pigs fed the PD diet had increased ($P < 0.05$) plasma $1,25(\text{OH})_2\text{D}_3$ and decreased ($P < 0.05$) plasma parathyroid hormone compared with those fed the PA diet. At the end of the trial, pigs fed the PD diet had decreased ($P < 0.05$) BW, HCW, and percentage fat-free lean and tended to have decreased LM area ($P = 0.06$) and marbling ($P = 0.09$) and greater ($P = 0.12$) 10th-rib backfat than pigs fed the PA diet. Additionally, animals fed the PD diet had weaker bones and also decreased ($P < 0.05$) ash percentage and increased ($P < 0.05$) concentrations of 1α -hydroxylase and parathy-

roid hormone receptor mRNA in kidney tissue. Regardless of dietary treatment, PIC337-sired pigs consumed more feed and gained more BW than their PIC280-sired counterparts ($P < 0.05$) during the study. The PIC337-sired pigs also had greater ($P < 0.05$) HCW, larger ($P < 0.01$) LM area, and tended to have ($P = 0.07$) greater dressing percentage. Meat from the PIC337-sired pigs also tended to have greater ($P = 0.12$) concentrations of lactate but decreased ($P = 0.07$) concentrations of total glucose units 24 h postslaughter. Although plasma $1,25(\text{OH})_2\text{D}_3$ concentrations were elevated ($P < 0.05$) in all the animals fed the PD diet, this elevation due to P deficiency tended ($P = 0.09$) to be greater in the PIC337-sired pigs after 12 wk on the treatment. The PIC337-sired pigs had stronger ($P < 0.01$) bones with greater ash percentage than the PIC280-sired pigs. The difference in the strength of the radii between the PIC337-sired pigs fed the PA and PD diets was greater than their PIC280-sired counterparts, which resulted in sire line \times treatment interactions ($P < 0.05$). These data indicate differing mechanisms of P utilization between these genetic lines. Elucidating these mechanisms may lead to strategies to increase efficiency of growth in a more environmentally friendly manner.

Key words: bone, meat quality, phosphorus, pig

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INTRODUCTION

Although dietary P is essential for the growth, development, and maintenance of both muscular and

skeletal tissues, increased public and governmental concerns over the environmental effect of excess P in the excreta of pigs has driven research to minimize the environmental effect of swine production (Baxter et al., 2003; Sutton and Richert, 2004). These concerns have led to research focused on more accurately defining the P requirements of pigs, as well as developing and evaluating technologies that could minimize P excretion from pig production (Cromwell et al., 1995; Spencer et al., 2000; Veum et al., 2001; Jendza et al., 2005). Although there is a great deal of interest in reducing P excretion by production animals, such efforts could re-

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²Corresponding author: chad_stahl@ncsu.edu

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sult in subtle dietary P deficiencies that not only affect growth but also adversely affect bone integrity. The prevention of dietary P deficiency is critical to maintaining the profitability of animal agriculture as well as animal well-being.

Very little work has examined the influence of genetics, with the exception of genetic P regulatory diseases, on P nutrition in any species. Hittmeier et al. (2006) demonstrated that the metabolic response to a severe dietary P deficiency was modulated by genetic background in young pigs. Interest in further reducing the environmental effect of swine production has fueled the need for a better understanding of the effects of subtle P deficiency and how genetics may mediate these effects. The objective of this study was to examine the effect of genetic background on the effects of long-term, subtle dietary P deficiency on growing pigs with the same genetic backgrounds utilized by Hittmeier et al. (2006).

MATERIALS AND METHODS

All animal protocols were approved by Iowa State University's Institutional Animal Care and Use Committee.

Animals

Ninety-four young female pigs at 28 ± 2 d of age (8.6 ± 1.3 kg) were obtained by breeding sows (Camborough 22, Pig Improvement Corporation, Franklin, KY) with mixed semen from 1 of 2 genetic lines known to differ in bone weight and lean growth potential (PIC337 and PIC280, Pig Improvement Corporation). The PIC280 line is Duroc-based, and the PIC337 line is a synthetic white line. Carcasses of PIC280 pigs have greater bone weight in all of the primal cuts compared with carcasses from PIC337 pigs. The gilts were housed individually in 0.5-m^2 pens under controlled environmental conditions (23 to 26°C and 14 h of light/d). All piglets were given ad libitum access to a basal diet, which met or exceeded their nutrient requirements (NRC, 1998) for 1 wk before the beginning of the trial to allow them to acclimate to their new environment. All pigs were weighed and allotted on the basis of litter and BW to 1 of 2 dietary treatment groups. All animals had ad libitum access to water and either a P-adequate (PA) or a 20% P-deficient (PD) diet (Table 1) over the 14-wk trial. A total of 3 pigs were removed from the study because of illnesses unrelated to genetic background or dietary treatment. Diets were formulated based on NRC (1998) recommendations and were reformulated 4 times over the course of the study to reflect the changing dietary requirements of the growing pig (Table 1). Body weight and feed intake were recorded monthly after an overnight fast throughout the trial. Blood samples (3 mL) were also collected monthly by venipuncture of a jugular vein using heparinized tubes (Vacutainer Plus, BD Vacutainer, Franklin Lakes, NJ), and plasma was ob-

tained by centrifugation at $3,500 \times g$ and 4°C . Plasma samples were stored at -20°C until analysis.

Upon completion of the study, all animals were processed under USDA inspection at the Iowa State University Meat Laboratory. Pigs were electrically stunned using a head-only electric stun tong apparatus (BTR 100 AVS, Freund Maschinenfabrik GmbH & Co. KG, Paderborn, Germany), subsequently exsanguinated, scalded, and mechanically dehaired in a scald-dehairing tank (Oscar Baumann GmbH Co. type BM 20, Pioneer Food Equipment, Penns Grove, NJ) and then eviscerated and weighed. An approximately 1×2.5 cm sample of cortex tissue from the right kidney was collected from each pig and snap-frozen in liquid N and stored at -80°C until RNA extraction for gene expression analysis. Radial bones with attached ulnae and intermediate carpals were collected and stored at 4°C for bone strength analysis and ash percentage, respectively. The carcasses were placed in an air-chilling system at 4°C for 24 h. After chilling, 2 LM chops from the left side of each carcass were collected for meat quality and glycolytic potential analyses.

An additional 27 female pigs produced by the same breeding strategy (Camborough 22 \times PIC337 or PIC280) were group-housed and fed the same treatment diets (PA or PD) described before. After 12 wk on the experimental diets (approximately 100 kg of BW), pigs were moved into individual stainless steel metabolism crates (1.2×2.4 m) and adapted to both the metabolism crates and a twice-daily feeding regimen for 3 d before a 5-d total fecal and urine collection period. During the metabolism trial, 1.2 kg of the respective treatment diet was fed at both 0700 and 1900 h, withorts subtracted from total feed intake to determine actual feed consumed. Total P intake was calculated from actual feed consumption and the analyzed diet composition. Water was supplied ad libitum through nipple waters. Twice daily, feces and urine from each metabolism crate were collected and frozen at -20°C until further analysis. Ambient temperature in the metabolism room was maintained at approximately 22°C , and lighting was provided continuously.

Plasma Analysis

Calcium, P, $1,25(\text{OH})_2\text{D}_3$, parathyroid hormone (PTH) concentrations (the minimum level of detection was 1 pg/mL; intraassay CV $< 10\%$), as well as alkaline phosphatase (ALP) activity were determined for all plasma samples. Plasma Ca concentrations were determined using a Cole-Palmer digital flame analyzer (model 2655-00, Cole-Palmer Instrument Co., Chicago, IL). Inorganic P concentrations were determined by the method of Gomori (1942) modified for use with a microplate spectrophotometer (PowerWave HT Microplate Scanning Spectrophotometer, Bio-Tek, Winooski, VT). Briefly, plasma was deproteinized with 12.5% trichloroacetic acid and assayed using Elon solution (*p*-methylaminophenol sulfate). The concentra-

Table 1. Experimental diet composition (as-fed basis)¹

Item	Diet 1		Diet 2		Diet 3		Diet 4	
	PA ²	PD ³	PA	PD	PA	PD	PD	PA
Ingredients, %								
Plasma, spray-dried	5	5	1.5	1.5	0	0	0	0
Corn	66.7	66.8	72.5	72.6	78.1	78.2	80.6	80.5
Soybean meal, 48% CP	23.5	23.5	22	22	19	19	17	17
Dicalcium phosphate	1.25	0.85	0.8	0.55	0.73	0.5	0.27	0.53
Limestone	1.15	1.39	0.97	1.12	0.9	1.05	0.85	0.71
Corn oil	1	1	1	1	0	0	0	0
Lysine-HCl	0.15	0.15	0	0	0	0	0	0
Methionine	0.05	0.05	0	0	0	0	0	0
Vitamins-minerals ⁴	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Antibiotics ⁵	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Analyzed ⁶								
CP, %	20.2	20.5	17.3	17.5	15.4	15.7	14.3	15.7
ME, kcal/kg	3,085	3,036	3,177	3,151	3,124	3,153	3,162	3,158
Calcium _{total} , %	0.99	0.94	0.66	0.64	0.64	0.58	0.58	0.54
Phosphorus _{total} , %	0.62	0.54	0.51	0.46	0.46	0.42	0.44	0.38

¹Fed diets 1, 2, 3, and 4 from 0 to 4, 4 to 8, 8 to 12, and 12 to 14 wk, respectively.

²Phosphorus-adequate diet.

³20% P-deficient diet.

⁴Provided the following quantities per kilogram of diet: 6,614 IU of vitamin A, 1,653 IU of vitamin D₃, 33 IU of vitamin E, 0.03 mg of vitamin B₁₂, 9.9 mg of riboflavin, 49.6 mg of niacin, 26.5 mg of pantothenic acid, 10.5 mg of copper, 105 mg of iron, 36 mg of manganese, 90 mg of zinc, and 1.2 mg of iodine.

⁵Provided the following per kilogram of diet: 110 mg of oxytetracycline and 77 mg of neomycin sulfate per kilogram of diet.

⁶Diets were analyzed by a commercial laboratory (Eurofins, Des Moines, IA).

tions of 1,25(OH)₂D₃ in the plasma samples were determined utilizing a commercially available EIA kit (IDS, Fountain Hills, AZ). The PTH plasma concentrations were determined using a porcine intact PTH ELISA kit (Immutopics, San Clemente, CA). Alkaline phosphatase activity was assayed by the method of Bowers and McComb (1966), in which the rate of formation of yellow-colored structures by the hydrolysis of *p*-nitrophenol phosphate to *p*-nitrophenol is proportional to the level of ALP activity in the plasma. The rate of appearance of this yellow color was determined at 405 nm.

Bone Measures

The radii with attached ulnae were manually cleaned of all soft tissue and utilized for flexural testing with an instrument (Instron Universal Testing Machine Model 4502, Instron, Canton, MA) equipped with a 10-kN load cell and configured for 3-point bending tests. Load applied at both yield and failure was determined using software (Series IX, v 8.08.00 software, Instron). The radii were placed on upright supports spaced 3 cm apart, and the crosshead applied pressure to the bone equidistant between the 2 uprights. The crosshead speed was set at 50 mm/min. Mineral content of the intermediate carpals was determined by drying at 110°C for 24 h followed by ashing at 600°C for 24 h.

Real-Time PCR

Total RNA was isolated from kidney tissue (RNeasy Midi Kits, Qiagen, Valencia, CA) according to the in-

structions of the manufacturer. Genomic DNA contamination was then removed from the isolated RNA by treatment with deoxyribonuclease (DNA-free kit, Ambion, Austin, TX). The RNA was then reverse-transcribed with Superscript II (Invitrogen Life Technologies, Carlsbad, CA) according to the instructions of the manufacturer, and the resulting cDNA samples were then treated with RNase H (Invitrogen) to ensure the removal of residual RNA. Primer sets for vitamin D receptor, calcitonin receptor, sodium-phosphate cotransporter 2 (**NPT2**), PTH receptor (**PTHr**), and 1 α -hydroxylase (**CYP27B1**) mRNA were designed using software (Integrated DNA Technologies, Coralville, IA; Table 2). Primers were validated according to the specifications set forth by Livak and Schmittgen (2001). Relative quantities of the transcripts of interest were determined by semiquantitative real-time PCR (MyiQ Single Color Real-Time PCR Detection System and SybrGreen Supermix, Bio-Rad Laboratories, Hercules, CA). Thermocycling conditions included 40 cycles of 30 s of melting at 95°C followed by 30 s of annealing and extension at 60°C. After amplification, all samples were subjected to a melt curve analysis. Gene expression was normalized to cDNA concentration as determined by Picogreen (Invitrogen) according to the instructions of the manufacturer.

Carcass Characteristics

Carcasses were weighed at slaughter to determine HCW, which was used to calculate percentage of fat-

free lean (FFL) and dressing percentage. The pH of the LM was measured after 24 h of chilling. The pH was determined using a pH meter (Hanna 9025 pH/ORP meter, Hanna Instrument, Woonsocket, RI). It was calibrated at 4°C with 2 pH calibration solutions (7.080 ± 0.002 and 4.667 ± 0.006) before data collection. The pH of the LM was obtained by insertion of the pH probe between the 12th and 13th ribs of the left side of the carcass.

Meat Quality

After chilling the carcass for 24 h, two 1.5-cm-thick LM chops were obtained immediately distal to the 13th rib. With these muscle samples, drip loss and color were measured. Drip loss was determined as the amount of purge that resulted during the storage of the chop for 24 h at 4°C (Lonergan et al., 2001). Color values were determined at 1 d postmortem. Boneless LM chops were allowed to bloom for 1 h at room temperature, and color values were determined (Hunter Labscan colorimeter, Hunter Association Laboratories Inc., Reston, VA). A CIE D/65 10° standard observer and a 2.54-cm viewing port were used to obtain 3 color measurements on each of 2 chops. All 6 color measurements were used to determine an average color score for each LM. The L* (greater value indicates a lighter color), a* (greater value indicates a redder color), and b* (greater value indicates a more yellow color) values were measured in 3 replicate measurements on each muscle sample, resulting in 6 measurements per muscle.

Glycolytic Potential

The chops used for the drip loss and color measurements were frozen at -20°C until analysis for glycolytic potential. Total micromolar glucosyl units (glucose, glucose-6-P, and glucose from glycogen) were determined with the modified protocol of Monin and Sellier (1985). Glycolytic potential units (μmol/g of LM) were calculated as follows: glycolytic potential = 2([glycogen] + [glucose] + [glucose-6-P]) + [lactate].

P-Balance Analysis

After the P-balance study, the feeds utilized and feces collected were dried at 70°C for 24 h and ground through a 2-μm screen (ZM 100, Retsch GmbH, Haan, Germany). These samples, as well as the urine samples, were microwave-digested (Mars 5, CEM Corporation, Matthews, NC), solubilized with water (50-fold dilution), and analyzed for P using spectroscopy (inductively coupled plasma spectroscopy, Optima 5300DV, PerkinElmer, Shelton, CT).

Statistics

Data were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC) with sire line, dietary treatment, and the interaction of the 2 considered as fixed effects. Sires could not be included as a fixed effect because mixed semen were used. Initial BW was used as a covariate for growth performance data, whereas final BW was utilized as a covariate for bone biomechanical data, carcass characteristics, meat quality, and glycolytic potential.

RESULTS

Plasma

Initially, there was a sire line effect ($P < 0.05$) on plasma P concentrations, with the PIC280-sired animals having greater concentrations than their PIC337-sired counterparts (8.0 vs. 7.6 mg/dL, respectively). No sire line or sire line × dietary treatment interactions were observed at any other time during the study. After 4 wk on trial, pigs fed the PD diet had decreased ($P < 0.05$) plasma P concentrations than those fed the PA diet (Figure 1). This treatment effect remained for the duration of the trial. There were no differences in plasma concentrations of either 1,25(OH)₂D₃ or PTH among any of the groups at the beginning of the study. A dramatic increase ($P < 0.05$) in plasma PTH concentrations was detected at 4 wk in pigs fed the PA diet (Figure 2). This difference was not apparent at 12 wk, and the concentrations of PTH in all animals were very

Table 2. Primers used for quantification of gene expression by real-time PCR

Gene name	Primer sequence ¹
Sodium phosphate cotransporter 2	F: 5' TGATGCTTGCCTTCCTCTACCTCT 3' R: 5' ACAGGATGGCGTTGTCCTTGAAGA 3'
Vitamin D receptor	F: 5' TTGCCAAACACCTCAAGCACAAAG 3' R: 5' TGCTCTACGCCAAGATGATCCAGA 3'
Calcitonin receptor	F: 5' TGCTCATGCCATTACTAGGGCAGT 3' R: 5' ATGACAGGGCCGTGGATGATGTAA 3'
Parathyroid hormone receptor	F: 5' TACTGTTTCTGCAACGGCGAGGTA 3' R: 5' GCGCTTAAAGTCCAGTGCCAATGT 3'
1α-Hydroxylase	F: 5' AGGAGTGAAGTATGCACTTGGCCT 3' R: 5' GGAGCGGCCCAAGAAATAGCAAA 3'

¹F = forward; R = reverse.

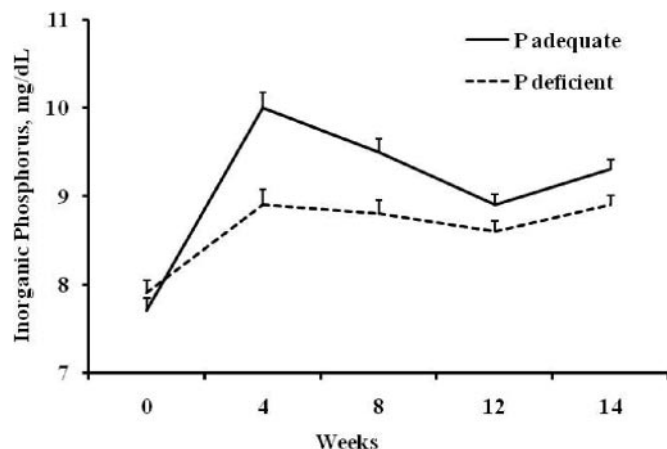


Figure 1. Effect of dietary P on plasma inorganic phosphate concentrations (mg/dL). Values presented are least squares means and SE. Differences between dietary treatment groups from 4 to 14 wk: $P < 0.05$. P adequate, $n = 45$; P deficient, $n = 46$.

low. Plasma concentrations of $1,25(\text{OH})_2\text{D}_3$ were 1.2 and 1.3 times greater in pigs fed the PD diet than those fed the PA diet at 4 wk ($P < 0.01$) and 12 wk, respectively (Figure 3). At 12 wk, there was a trend ($P = 0.09$) for the interaction of sire line and dietary treatment on plasma concentrations of $1,25(\text{OH})_2\text{D}_3$. The interaction was the result of decreased plasma concentrations of $1,25(\text{OH})_2\text{D}_3$ in pigs sired by PIC337 when fed the PA diet compared with their PIC280-sired counterparts. This resulted in differences in plasma $1,25(\text{OH})_2\text{D}_3$ between pigs fed the PA diet and the PD diet that tended to be greater in the PIC337-sired pigs. There were no differences in plasma ALP activity or Ca concentrations among any of groups throughout the study (data not shown).

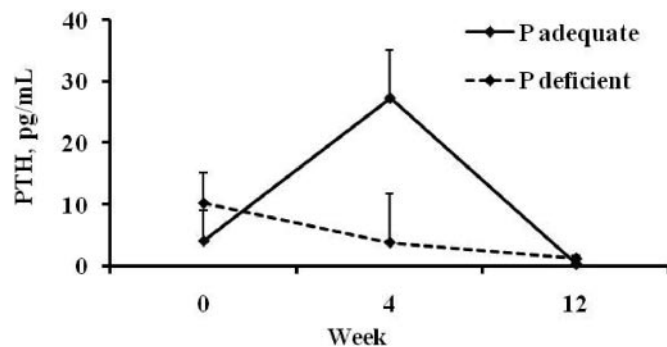


Figure 2. Effect of dietary P on plasma parathyroid hormone (PTH) concentrations (pg/mL). Values presented are least squares means and SE. Differences between dietary treatment groups at 4 wk: $P < 0.05$. P adequate, $n = 45$; P deficient, $n = 46$.

Growth Performance

Over the entire study, the PIC337 progeny had greater ($P < 0.05$) ADG and consumed more ($P < 0.05$) feed than their PIC280-sired counterparts (Table 3). Dietary P levels did not affect overall ADG or feed intake. There was a trend ($P = 0.12$) for an interaction between sire line and dietary P levels affecting overall ADG, with the ADG of PD-fed, PIC337-sired pigs being decreased compared with their PA-fed littermates. There was no effect of the sire line or a sire line \times dietary treatment interaction on overall G:F; however, it was decreased ($P < 0.05$) in pigs fed the PD diet. During the first 4 wk of the study, both dietary P level and the interaction between sire line and dietary P level affected ($P < 0.01$) G:F of these pigs. These seem to be the result of the PIC337-sired pigs fed the PA diet having the greatest G:F and a decrease in G:F with dietary P deficiency observed only in the PIC337-sired animals.

Bone Characteristics

Pigs fed the PD diet had reduced bone strength and ash percentage ($P < 0.01$; Figure 4). The subtle P deficiency reduced the maximum load tolerated by the radii by approximately 16%, load at bone failure by approximately 28%, and reduced bone ash percentage by approximately 6%. The PIC337-sired pigs had stronger ($P < 0.01$) bones with greater ash percentage than the PIC280-sired pigs. Interactions ($P < 0.05$) between sire line and dietary P levels were observed in both the load at yield and load at failure measures of the radial bones, with a greater difference in the strength of the radii between PIC337-sired pigs fed the PA and PD diets as compared with their PIC280-sired counterparts.

Gene Expression in Kidney

The levels of PTHR and CYP27B1 mRNA were approximately 1.5- and 1.8-fold greater ($P < 0.05$), respectively, in the kidney tissue of pigs fed the PD diet than those fed the PA diet (Figure 5). No differences were observed in the expression of vitamin D receptor, calcitonin receptor, or NPT2 among any of the dietary treatment groups (data not shown).

P Balance

There were no differences between the 2 sire lines or interactions between the sire line and dietary P in any of the P-balance indices measured (Table 4). Animals fed the PD diet had decreased dietary P intakes, as well as decreased fecal P excretion, compared with pigs fed the PA diet ($P < 0.01$). Pigs fed the PD diet also tended ($P = 0.09$) to excrete less P in their urine. There were no effects of dietary P on P digestibility or retention when expressed on a gram or percentage of intake basis.

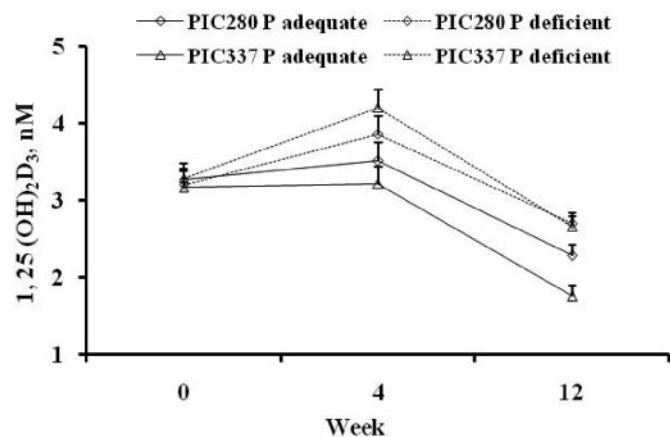


Figure 3. Effect of dietary P and genetic background interactions on plasma 1,25(OH)₂D₃ concentrations. The PIC280-sired line is a Duroc-based heavier-boned line. The PIC337-sired line is a synthetic white line that is lighter-boned. Values presented are least squares means and SE. Differences between dietary treatment groups at 4 wk: $P < 0.05$. Effects at 12 wk: sire line, $P < 0.05$; treatment, $P < 0.05$; and sire line \times treatment interaction, $P = 0.09$. PIC280-P deficient, $n = 23$; PIC280-P adequate, $n = 22$; PIC337-P deficient, $n = 23$; PIC337-P adequate, $n = 23$.

Carcass Characteristics

Dietary P deficiency reduced BW, HCW, and carcass FFL ($P < 0.05$) and tended to reduce ($P = 0.06$) LM area (Table 5). A trend toward pigs fed the PD to have a greater depth of 10th-rib backfat ($P = 0.12$) was observed. The PIC337-sired pigs had larger LM area

and greater HCW ($P < 0.05$) and tended to have greater dressing percentage ($P = 0.07$) than their PIC280 counterparts. Body weight, carcass FFL, and 10th-rib backfat were not affected by genetic background. No sire line \times dietary treatment interaction was observed for any of the carcass characteristics measured.

Meat Quality

Despite having less backfat, pigs fed the PA diet tended to have greater LM marbling scores than those fed the PD diet ($P < 0.1$; Table 6). There was no effect of genetic background observed for any meat quality measurement. The only measure that was affected by the interaction of dietary P level and genetic background was the b^* score ($P < 0.05$). The PIC337-sired pigs fed the PA diet had lower b^* score compared with those fed the PD diet, whereas b^* score was not affected by dietary treatment in PIC280-sired pigs. Both treatment groups of PIC280-sired pigs had b^* scores that were similar to the PD-fed, PIC337-sired pigs.

Glycolytic Potential

There was no effect of dietary treatment on any of the glycolytic potential measurements (Table 7). There was a trend for the effect of genetic background on 24-h total glucose ($P = 0.07$) and 24-h total lactate ($P = 0.12$) but no effect on glycolytic potential. The PIC337-sired pigs tended to have decreased 24-h total glucose unit and greater 24-h total lactate compared with PIC280-sired pigs ($P = 0.12$). No sire line \times dietary treatment interactions were observed for any of the glycolytic potential measurements.

Table 3. Effect of dietary P and genetic background on growth performance¹

Item	PIC280 ²				PIC337 ³				<i>P</i> -value		
	PA ⁴	SE	PD ⁴	SE	PA	SE	PD	SE	T ⁵	S ⁵	T \times S
ADG, kg											
0 to 4 wk	0.562	0.013	0.560	0.013	0.600	0.013	0.568	0.013	0.17	0.08	0.23
4 to 8 wk	0.869	0.018	0.849	0.017	0.890	0.017	0.840	0.017	0.04	0.72	0.38
8 to 12 wk	0.927	0.022	0.960	0.022	0.990	0.022	0.974	0.022	0.68	0.09	0.26
Overall ⁶	0.837	0.013	0.848	0.012	0.887	0.013	0.860	0.012	0.54	0.02	0.12
ADFI, kg											
0 to 4 wk	0.860	0.019	0.863	0.019	0.882	0.019	0.893	0.019	0.73	0.17	0.82
4 to 8 wk	1.85	0.036	1.90	0.035	1.88	0.035	1.85	0.035	0.87	0.80	0.26
8 to 12 wk	2.43	0.055	2.55	0.054	2.67	0.054	2.67	0.054	0.26	<0.01	0.30
Overall	1.89	0.034	1.93	0.032	1.99	0.032	1.99	0.032	0.40	0.03	0.30
G:F, kg/kg											
0 to 4 wk	0.653	0.008	0.652	0.008	0.680	0.008	0.637	0.008	<0.01	0.46	<0.01
4 to 8 wk	0.470	0.006	0.447	0.006	0.473	0.006	0.454	0.006	<0.01	0.40	0.74
8 to 12 wk	0.385	0.007	0.382	0.007	0.376	0.007	0.370	0.007	0.51	0.14	0.87
Overall	0.444	0.004	0.438	0.004	0.445	0.004	0.434	0.004	0.02	0.68	0.59

¹Values presented are least squares means and standard errors. For all groups, $n = 23$, except for PIC280/PA where $n = 22$.

²PIC280 = heavier-boned genetic line.

³PIC337 = lighter-boned genetic line.

⁴PA = P adequate; PD = P deficient.

⁵T = dietary treatment; S = sire line.

⁶0 to 14 wk.

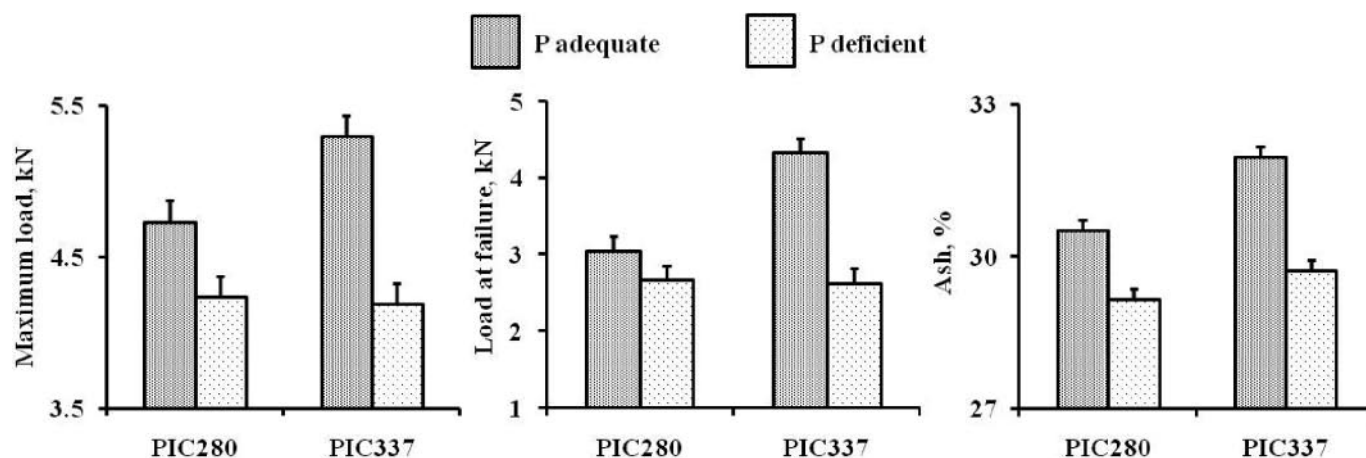


Figure 4. Effect of dietary P and genetic background interactions on bone strength. The PIC280-sired line is a Duroc-based heavier-boned line. The PIC337-sired line is a synthetic white line that is lighter-boned. Values presented are least squares means and SE. Maximum load: sire line, $P = 0.07$; treatment, $P < 0.01$; and sire line \times treatment interaction, $P < 0.05$. Load at failure: sire line, $P < 0.01$; dietary treatment, $P < 0.01$; and their interaction, $P < 0.05$. Ash percentage: sire line, $P < 0.01$ and treatment, $P < 0.05$. PIC280-P deficient, $n = 23$; PIC280-P adequate, $n = 22$; PIC337-P deficient, $n = 23$; PIC337-P adequate, $n = 23$.

DISCUSSION

The objective of this study was to evaluate the effect of genetic background on the responses to subtle dietary P deficiency in growing pigs. This mild deficiency was designed to represent a plausible dietary situation in commercial pig production. The treatment diets achieved the desired level of deficiency as evidenced by the slightly decreased plasma P concentrations (Figure 1) and reduced bone integrity and mineral content (Figure 4), but with a lack of a strong growth depression (Table 3) or a change in P retention (Table 4)

among pigs fed the PD diet. Although the effects of dietary P deficiency in growing pigs have been described previously (Cromwell et al., 1995; Spencer et al., 2000; Veum et al., 2001; Jendza et al., 2005), the interaction between dietary P level and genetic background has received very little attention.

As expected, pigs fed the PD diet had weaker bones with decreased mineral content compared with their counterparts fed the PA diet. Interestingly, genetic background had an effect on the effect of dietary P deficiency on measures of bone integrity (Figure 4). The response of bone to dietary P deficiency was more dra-

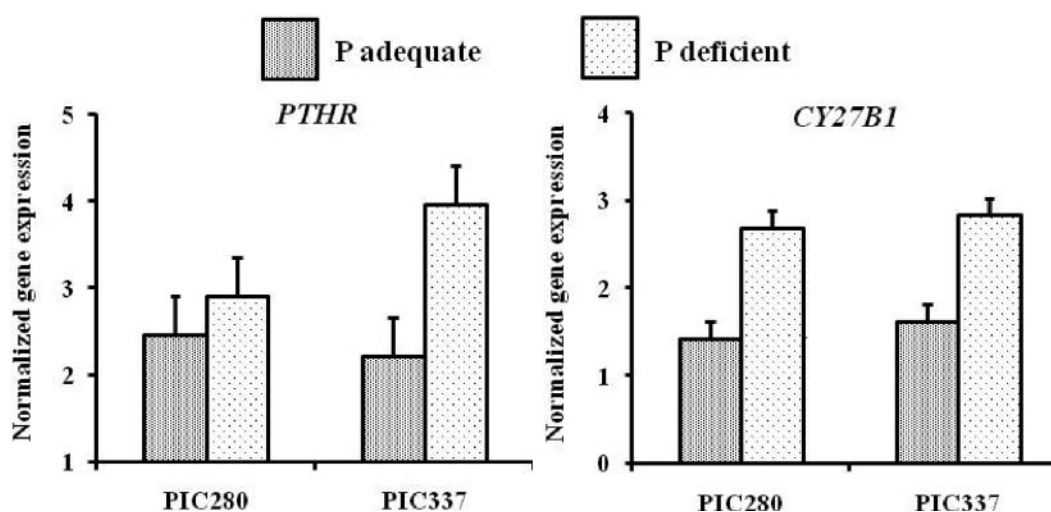


Figure 5. Effect of dietary P on parathyroid hormone receptor (PTHr) and 1 α -hydroxylase (CYP27B1) mRNA expression in kidney tissue. The PIC280-sired line is a Duroc-based heavier-boned line. The PIC337-sired line is a synthetic white line that is lighter-boned. Values presented are least squares means and SE. Gene expression was normalized to cDNA concentration as determined by Picogreen (Invitrogen, Carlsbad, CA). Treatment: PTHr, $P < 0.05$ and CYP27B1, $P < 0.05$. PIC280-P deficient, $n = 23$; PIC280-P adequate, $n = 22$; PIC337-P deficient, $n = 23$; PIC337-P adequate, $n = 23$.

Table 4. Phosphate balance indices of 2 lines of pigs fed diets adequate or deficient in phosphate¹

Item	PIC280 ²				PIC337 ³				P-value		
	PA ⁴	SE	PD ⁴	SE	PA	SE	PD	SE	T ⁵	S ⁵	T × S
Intake, g	43.25	1.02	37.35	0.94	43.46	0.94	36.52	0.94	<0.01	0.74	0.59
Fecal, g	23.31	1.28	19.74	1.18	24.55	1.18	19.08	1.18	<0.01	0.81	0.44
Digestible, g	19.94	1.04	17.62	0.97	18.91	0.97	17.43	0.97	0.07	0.54	0.67
Digestible, %	46.14	2.56	47.24	2.37	43.60	2.37	47.85	2.37	0.28	0.69	0.52
Urine, g	0.264	0.466	0.208	0.431	0.246	0.431	0.144	0.431	0.09	0.37	0.61
Retained, g	19.67	1.05	17.41	0.97	18.66	0.97	17.29	0.97	0.08	0.57	0.66
Retained, %	45.53	2.59	46.68	2.39	43.03	2.39	47.46	2.39	0.27	0.73	0.51

¹Values presented are least squares means. For all groups, n = 7, except for PIC280-PA where n = 6.

²PIC280 = heavier-boned genetic line.

³PIC337 = lighter-boned genetic line.

⁴PA = P adequate; PD = P deficient.

⁵T = dietary treatment; S = sire line.

matic among the PIC337-sired pigs. There tended to be a similar response in ADG over the entire study, despite the absence of a sire line × dietary treatment interaction effect on ADFI or G:F (Table 3). During the first 4 wk of this study, when the pigs would have had their greatest dietary requirement for available P on a percentage basis, dietary P deficiency decreased G:F, and there was an interaction of sire line with dietary treatment on G:F. The G:F of PIC337-sired pigs fed the PD diet was dramatically reduced, whereas the PIC280-sired pigs did not seem responsive to the dietary P restriction.

Although growth rate and efficiency, as well as bone strength and mineral content, are important measures of P status, to help understand the differences based on genetic background seen in these measures, circulating concentrations of the endocrine hormones known to be responsible for Ca and P homeostasis were examined. The effect of dietary P deficiency on circulating concentrations of PTH and 1,25(OH)₂D₃ has been described previously (Moallem et al., 1998; Riond et al., 2001; Yoshida et al., 2001; Zhang et al., 2002). Several studies have demonstrated marked increases in serum 1,25(OH)₂D₃ during P deprivation due to direct induction of CYP27B1 activity by dietary P (Yoshida et al.,

2001; Zhang et al., 2002). Riond et al. (2001) showed similar results, as well as a positive correlation between serum P and PTH concentrations. Additional research supported this correlation by establishing the role of decreased dietary P in reducing PTH mRNA expression in the parathyroid glands (Moallem et al., 1998). In the current study, PTH concentrations were almost undetectable in all groups at 12 wk. Dramatic suppression of circulating concentrations of PTH has been previously reported, which was attributed to the inhibition of PTH mRNA transcription by high concentrations of 1,25(OH)₂D₃ (Demay et al., 1992; Moallem et al., 1998; Darwish and DeLuca, 1999; Jaaskalainen et al., 2005). Although an increase in circulating concentrations of 1,25(OH)₂D₃ in pigs fed the PD diet was seen in our study, the magnitude of increase was not as pronounced as in the aforementioned studies (Moallem et al., 1998; Yoshida et al., 2001; Zhang et al., 2002). These other studies all imposed a far more severe P restriction than the present study, with virtually no P supplementation to the deficient diets. Of particular interest in this study were the differences in circulating concentrations of 1,25(OH)₂D₃ based on genetic background and the interaction of genetic background and dietary P. At 12 wk, PIC337-sired pigs had decreased

Table 5. Effect of dietary P and genetic background on carcass characteristics¹

Measurements	PIC280 ²				PIC337 ³				P-value		
	PA ⁴	SE	PD ⁴	SE	PA	SE	PD	SE	T ⁵	S ⁵	T × S
10th-rib backfat, cm	1.75	0.04	1.85	0.04	1.78	0.04	1.91	0.04	0.12	0.59	0.88
LM area, cm ²	35.48	0.42	33.61	0.39	37.94	0.39	36.77	0.39	0.06	<0.01	0.63
Live weight, kg	97.25	0.12	97.03	0.12	97.46	0.12	96.63	0.12	0.03	0.70	0.22
HCW, kg	70.18	0.27	69.24	0.27	71.83	0.27	70.21	0.27	0.02	0.02	0.53
Carcass fat-free lean, %	53.15	0.27	52.03	0.26	53.65	0.27	52.61	0.26	0.04	0.31	0.93
Dressing percentage, %	72.16	0.32	71.36	0.32	73.70	0.32	72.66	0.32	0.21	0.07	0.64
24-h pH	5.36	0.01	5.37	0.01	5.38	0.01	5.37	0.01	0.96	0.61	0.37

¹Values are least squares means. For all groups, n = 23, except for PIC280-PA where n = 22.

²PIC280 = heavier-boned genetic line.

³PIC337 = lighter-boned genetic line.

⁴PA = P adequate; PD = P deficient.

⁵T = dietary treatment; S = sire line.

Table 6. Effect of dietary P and genetic background on meat quality of the LM¹

Measurements	PIC280 ²				PIC337 ³				<i>P</i> -value		
	PA ⁴	SE	PD ⁴	SE	PA	SE	PD	SE	T ⁵	S ⁵	T × S
Marbling	1.89	0.07	1.68	0.07	1.86	0.07	1.63	0.07	0.09	0.79	0.94
Color	2.06	0.07	2.13	0.07	2.19	0.07	2.16	0.07	0.87	0.56	0.69
L*	61.97	0.39	61.96	0.37	60.92	0.38	62.02	0.37	0.47	0.51	0.47
a*	8.73	0.12	8.15	0.11	8.30	0.12	8.37	0.11	0.25	0.65	0.16
b*	15.29	0.12	15.01	0.12	14.54	0.12	15.22	0.12	0.40	0.26	0.05
Firmness	1.58	0.05	1.44	0.05	1.53	0.05	1.55	0.05	0.51	0.74	0.40
Wetness	1.68	0.05	1.59	0.05	1.55	0.05	1.53	0.05	0.57	0.31	0.70
Drip loss	3.66	0.13	3.38	0.12	3.53	0.12	3.40	0.12	0.40	0.82	0.75

¹Values are least squares means. For all groups, n = 23, except for PIC280-PA where n = 22.

²PIC280 = heavier-boned genetic line.

³PIC337 = lighter-boned genetic line.

⁴PA = P adequate; PD = P deficient.

⁵T = dietary treatment; S = sire line.

plasma concentrations of 1,25(OH)₂D₃ compared with their PIC280-sired counterparts. Additionally, there was an effect of the interaction between sire line and dietary treatment on plasma 1,25(OH)₂D₃ concentrations, with the magnitude of the increase observed with dietary P deficiency being greater among the PIC337-sired animals. These data indicate possible differences in hormone production based on genetic background (rather than diet alone) in maintenance of mineral homeostasis. This further validates our hypothesis that genetic background affects the regulation of P utilization.

Because the primary regulation of P homeostasis is thought to occur at the level of reabsorption by the kidneys, we examined the expression of several hormone receptors and enzymes involved in mediating the metabolic response to P deficiency. An important regulator of Ca and P homeostasis is the activation of 1,25(OH)₂D₃ by CYP27B1 in the kidney. The expression of CYP27B1 has been shown to be regulated by dietary P, PTH, and 1,25(OH)₂D₃ (Gonzalez and Martin, 1996; Takahashi et al., 1998; Kawane et al., 2001; Mulroney et al., 2004). With the greater circulating concentrations of 1,25(OH)₂D₃ observed among our PD animals, it was not surprising that we observed a similar increase in renal expression of CYP27B1 mRNA. Among pigs

fed the PD diet in the present study, the expression of PTHR mRNA was also elevated. Although this has not been reported previously, a possible explanation for the increased expression is that the suppression of PTHR expression by PTH (Gonzalez and Martin, 1996; Kawane et al., 2001) would likely not take place with the very low concentrations of circulating PTH seen at the end of this study. In addition to increases in the message for CYP27B1, levels of NPT2 mRNA have been shown to be dramatically increased during P deficiency (Takahashi et al., 1998; Mulroney et al., 2004). We did not see an increase in the expression of this gene based on dietary P restriction. It is possible that an increase in the gene expression of NPT2 was not observed in our study because our animals were meeting their daily P requirement at the time tissue was collected. The phase feeding and diet formulations utilized in this study were designed to produce a consistent, albeit mild, P deficiency. As the pigs grow, their dietary P requirements are reduced on a percentage basis. Therefore, toward the end of a feeding phase, they are less P deficient than they are at the beginning of the phase. Our analysis of gene expression in the kidney evaluated a single point in time at the end of our last feeding phase. Throughout the entire study, this would be the point where the pigs fed the PD diet

Table 7. Effect of dietary P and genetic background on glycolytic potential¹

Measurements	PIC280 ²				PIC337 ³				<i>P</i> -value		
	PA ⁴	SE	PD ⁴	SE	PA ⁴	SE	PD ⁴	SE	T ⁵	S ⁵	T × S
Lactate, μmol/g of tissue	125.26	1.40	124.91	1.39	127.07	1.38	131.90	1.35	0.41	0.12	0.35
Total glucose, μmol/g of tissue	21.12	0.77	20.51	0.74	18.01	0.75	18.02	0.74	0.84	0.07	0.84
Glycolytic potential, μmol/g of tissue	167.48	2.18	165.53	2.17	163.23	2.15	167.97	2.11	0.74	0.83	0.44

¹Values are least squares means. For all groups, n = 23, except for PIC280-PA where n = 22.

²PIC280 = heavier-boned genetic line.

³PIC337 = lighter-boned genetic line.

⁴PA = P adequate; PD = P deficient.

⁵T = dietary treatment; S = sire line.

would be the least P deficient. The lack of a difference in NPT2 mRNA in the kidney is supported by the absence of an increase in P retention among the PD pigs in our P-balance study (Table 4).

Although muscle tissue contains the second largest P pool in the animal, little research has examined the effect of genetic background on the response of carcass characteristics and meat quality during dietary P restriction. Similar to previous studies (Cromwell et al., 1970; Stockland and Blaylock, 1973; O'Quinn et al., 1997; Shelton et al., 2004), the present study did not observe an effect of P deprivation on carcass characteristics with the exception of BW, HCW, and carcass FFL percentage. Interestingly, even in slight P deficiency, pigs fed the PD diet tended to have greater 10th-rib backfat and smaller LM area when compared with their PA-fed counterparts. These trends are supported by previous studies (Cromwell et al., 1970; O'Quinn et al., 1997).

An effect of sire line on carcass characteristics was expected, because these 2 sire lines were originally selected for different traits. The effects of genetic background on the carcass characteristic measurements in this study can be easily explained by the different genetic lean growth potentials of the 2 sire lines. The differences observed between the genetic lines in the present study are supported by similar findings in work performed by the Pig Improvement Corporation. Those animals sired by PIC337 have less backfat thickness, increased lean percentage, and greater LM depth (A. A. Sosnicki, Iowa State University, Ames, personal communication).

The industrialization of animal agriculture has led to the creation of intensive production facilities to maximize the efficiency of animal production. This intensification has led to increased P runoff into the surrounding environment. Public concern over the environmental and economic effects of pollution stemming from animal agriculture has led to the development of various nutritional strategies that aim to reduce dietary P levels without altering animal performance. With the increasing pressure to reduce P excretion, it becomes more likely to have minor P deficiencies in growing pigs. The genetics utilized in this study likely represent 25% of the pigs produced in the United States annually (van der Steen et al., 2005). As we have demonstrated in this study, minor dietary P deficiency can affect bone integrity and carcass characteristics without a clear effect on the growth of the animal. In this study, the PIC337-sired animals grew faster and produced an improved carcass compared with the PIC280-sired pigs. However, these animals also showed the greatest sensitivity to P deprivation based on ADG, bone strength, and circulating concentrations of 1,25(OH)₂D₃. Differences such as these demonstrate the need to better define P needs based on genetic background. Understanding the underlying genetic mechanisms that regulate P utilization may lead

to novel strategies to produce more environmentally friendly pigs.

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